type double patenting as being unpatentable over claims 1 and 2 of U.S. patent 5,536,642 to Barbera-Guillem et al. Applicant respectfully submits that claim 1, as amended, is patentable over claims 1 and 2 of Barbera-Guillem et al for the reasons provided hereinafter with respect to the rejection of claim 1 under 35 USC 102(e) as being anticipated by Barbera-Guillem et al. Therefore, it is respectfully requested that this rejection be withdrawn.

Claim 1 has been rejected under 35 USC 102(e) as being anticipated by Barbera-Guillem et al, the Examiner stating that Barbera-Guillem et al teaches a method of determining the probability of metastasis by detecting the level of expression of the lymphoid gene product, IL-2R, thus meeting the claim limitations.

Claim 1 has also been rejected under 35 USC 103(a) as not being patentable over Mayer et al (Int. J. Oncology 3:368-373, 1993-IDS 23) or Kim et al (Proc. Am. Assoc. Cancer Res. 34:62, 1993-IDS 43), the Examiner stating that Mayer et al teaches that detection of the lymphoid gene product, lck transcripts was known to be a marker for metastasis of epithelial cells and that progression of colorectal cancer cells to a metastatic stage was known to be accompanied by expression of lymphocyte-specific genes; that Kim et al teaches the association between metastatic potential and expression of several lymphoid gene products; and that it would not have been unobvious to detect lymphoid gene expression within solid, non-lymphoid tumor cells to predict metastatic potential with a reasonable expectation of success given the correlations between metastasis and lymphoid gene expression taught by Mayer et al or Kim et al and their recognized status as metastatic markers.

As amended, claim 1 recites predicting the lymphotropic metastatic potential of a solid non-lymphoid primary tumor by determining for at least one product selected from the group

consisting of TCRβ, CD3, CD4, CD8, and ZAP 70 the percentage of cells of each of the samples which express said at least one product. Support therefor may be found in the last sentence in the last full paragraph on page 1, the third full paragraph on page 2, and at page 15, lines 21 and 22, as well as in Table 6 on page 23, of the specification. CTβ is TCRβ, as indicated at page 11, line 16, of the specification. For the reasons provided hereinafter, it is respectfully submitted that claim 1, as amended, is not anticipated by and is unobvious over the references of record and is therefore patentable.

In accordance with the present invention, a plurality of representative samples of a solid non-lymphoid primary tumor are obtained and the percentage of cells of each of the samples which express one or more of certain products associated with the T-cell either as a T-cell receptor or portion thereof or cluster of differentiation or signal transduction molecule is determined in order to predict the lymphotropic metastatic potential of the tumor. If no tumor cells in all of the samples are detected to express such product or products, the metastatic potential of the as yet nonmetastasized primary tumor is predicted to be low. If a high percentage of the tumor cells in at least one of the samples is detected to express such product or products, the metastatic potential of the as yet nonmetastasized primary tumor is predicted to be high.

Five specific ones of the above T-cell associated products have been identified by both clinical and experimental testing (as discussed more specifically below) to show a high correlation between future metastasis when present in cancer cells and future non-metastasis when not found to be present in cancer cells. These products, TCRβ, CD3, CD4, CD8, and ZAP-70, are thus high predictors of metastasis when found in as yet nonmetastasized cancer cells. All of these products are associated with the T

cell receptor. Thus, more specifically, TCRβ (or CTβ) is the constant region of the β chain of the T cell receptor, an integral membrane protein expressed on the surface of T lymphocytes and occurring as a disulfide linked heterodimer that is non-covalently associated with CD3 chains (see Fig. 1). ZAP-70 is a T cell derived signal transduction molecule. CD3, CD4, and CD8 are clusters of differentiation of human leukocyte antigens. This predictability thus confirms Applicants' belief that the acquisition of lymphotropic metastasis by cancer cells is accompanied by their ability to express aberrant lymphoid specific genes or their products, i.e., a relation between T cells (which are always migratory) and cancer cells which become metastatic (invasive cancer cells which become migratory). See page 1, last full paragraph, of the specification. Thus, by looking for and finding one or more of such products associated with the T cell in a cancer cell, one can predict metastasis of a primary (as yet nonmetastasized) tumor.

Experimental Data

The following experimental data relies in part on testing on a human cell line, SW480, from a primary tumor, and its related cell line, SW620, which is from a metastasized tumor in the same patient a year later, and clones or subsets SW480E (has high metastatic potential) and SW480R (has low metastatic potential). See the paragraph which spans pages 10 and 11 of the specification. Also see Yoon et al, Abstract 3638, *Proceedings of the American Association for Cancer Research*, vol. 38, p. 542, March, 1997 (copy enclosed) wherein it is stated that the results of their study suggest that, even though the R-type (SW480R) cells are more tumorigenic, E-type (SW480E) cells are more invasive and metastatic. It is believed that, once a cell line such as SW620 has already metastasized or colonized at a distant

site, it may lose its ability to metastasize further.

Fig. 9 (see discussion thereof on page 11, first full paragraph, of the specification) contains data which shows that TCRβ (CTβ) was detected in the cloned tumor cell line, SW480E, which is metastasizing, and the amount detected was greater than the amount detected in the cloned tumor cell line, SW480R, which is nonmetastasizing, thus showing experimentally that, although TCRβ may sometimes be found in nonmetastasizing tumors, still the presence of TCRβ in cancer cells may be a high predictor of metastasis. The clinical data discussed hereafter will more clearly show that the presence of TCRβ may be a good predictor of metastatic potential.

Table 3 on page 20 of the specification contains data from experiments with Wistar Furth rats which shows that all of those breast cancer tumors (MT-449, MT-450, SMT-2A, and TMT-081) which were found to have cells expressing either CD4 or CD8 were metastasizing while all 6 tumors in which no CD4 or CD8 was found were nonmetastasizing. This data thus show experimentally that the presence of CD4 or CD8 in cancer cells may be a good predictor of metastasis.

Fig. 18 and Table 5 on page 22 of the specification (see the first two full paragraphs on page 13 of the specification) contain experimental data which shows that ZAP-70 was found to be present in SW480E cells, which are metastasizing, while little or none was found in the already metastasized cells, SW620, and that none was found in SW480R cells, which are nonmetastasizing, thus showing experimentally that the presence of ZAP-70 in cancer cells may be a good predictor of metastasis.

Clinical Data

Table 6 on page 23 of the specification shows the presence or absence of finding of the T-cell products CD3, CD4, CD8, and

TCRβ (CTβ) in fresh human breast cancer cells 13 of which had no lymph node involvement and were nonmetastasizing and 7 of which had lymph node involvement and were metastasizing. As seen in Table 6, with only an exception for one nonmetastasizing tumor wherein the presence of CD8 was positive and the presence of CD4 was borderline, none of the T-cell products CD3, CD4, CD8, and TCRβ (CTβ) was detected in any of the other 12 nonmetastasizing tumors and one or more of the T-cell products CD3, CD4, CD8, and TCRβ (CTβ) was detected in each of the 7 metastasizing tumors.

These clinical findings, as corroborated by the experimental data, clearly show that the detection of the presence of the T-cell associated products CD3, CD4, CD8, TCRβ (CTβ), and ZAP-70 in a solid, non-lymphoid primary tumor may be a predictor of metastasis and that their absence (non-detection) may be a predictor of nonmetastasis.

Barbera-Guillem et al

Barbera-Guillem et al discloses the use of measurement of cell-associated interleukin-2 receptor alpha (IL-2R alpha) expression in solid, non-lymphoid tumors in prognosing metastatic potential of the tumor and in monitoring the efficacy of anticancer therapy against metastatic cells of non-lymphoid tumors. As indicated at col. 1, last line, to col. 2, line 2, IL-2R alpha is expressed on activated T cells and exerts growth promoting effects on the T cell. Barbera-Guillem et al also discloses that human and experimental non-lymphoid tumors express TCRβ and that clinical applications of the cell-associated expression of TCRβ by human solid non-lymphoid tumors are (1) immune modulation, and (2) TCRS-targeted antineoplastic drug therapy including the use of the tumor specific TCRβ phenotype in monitoring the efficacy of anticancer treatment against non-lymphoid tumors. See col. 17, lines 33 to 35, and col. 18, line

17, of this reference.

While IL-2R may aid in promoting growth of the T-cell, it is not involved in giving the T-cell its characteristic of motility, which of course is also a necessary characteristic of a cancer cell for metastasis. The T-cell associated products, CD3, CD4, CD8, TCRβ (CTβ), and ZAP-70, are, on the other hand, involved in giving to the T-cell this characteristic of motility. These are thus products that should, in accordance with the theory behind the present invention, more clearly correlate with metastasis, and the experimental and clinical results discussed above do more clearly show that the T-cell related products in accordance with the present invention do more clearly correlate with metastasis (as compared with IL-2R alpha, wherein in Table 1 of Barbera-Guillem et al, it is shown that IL-2R alpha was not found in the metastasizing SW480E cells and was found in the nonmetastasizing SW480R cells; in reading Table 1 of Barbera-Guillem et al, it should be noted that the above Yoon et al Abstract, which does not contradict Tomita et al (IDS 30), states that the SW480E cells are more invasive and metastatic).

Although the use of TCRβ is taught as useful for other purposes therein, Barbera-Guillem et al does not disclose, teach, or suggest the use of the detection of TCRβ (or of CD3, CD4, CD8, or ZAP-70) as a predictor of metastasis.

Mayer et al

Mayer et al discloses that detection of *lck* transcripts, which are involved in signal transduction from the T cell receptor complex to the nucleus, has been discussed as a marker for metastasis of epithelial tumor cells and that progression of colorectal cells to a metastatic stage is often accompanied by the ability to express lymphocyte-specific genes.

Mayer et al appears to be confirmed by McCracken et al, "*An*

Alternative Pathway for Expression of p56lck from Type I promoter Transcripts in Colon Carcenoma," Oncogene, vol. 15, p. 2929-2937, 1997 (copy enclosed) due to its statement at page 2930 (and also similarly at page 2934) that, based on certain results, "in closely matched human colon carcinoma lines lck expression in vitro appears to correlate with tumorigenic or metastatic potential in vivo." This statement incorrectly assumes that tumorigenic potential is the same as metastatic potential. As discussed above, the Yoon et al Abstract (cited above and enclosed) clearly indicates that there is a difference between tumorigenic potential and metastatic potential, i.e., that even though the R-type (SW480R) cells are more tumorigenic, E-type (SW480E) cells are more invasive and metastatic (a finding which does not contradict Tomita et al (IDS 30)). Disregarding this incorrect conclusion in McCracken et al, the analysis in McCracken et al clearly shows that lck is not a good predictor of metastatic potential, i.e., as discussed in the previous sentence on page 2930 of McCracken et al, lck was more abundantly expressed in the already metastasized SW620 cell line and the nonmetastasizing SW480R (more specifically SW480R2) subset than in the primary SW480 cell line and the potentially metastatic SW480E (more specifically SW480E8) subset. Thus, the presence of *lck* in a tumor does not correlate with metastatic potential, and, accordingly, the detection of its presence in a tumor is not a predictor of metastasis.

Moreover, Mayer et al does not teach or suggest the use of detection of its lck marker as a predictor (future tense) that a cancer will metastasize. The use of detection of a marker to determine that a cancer has metastasized (present tense), as in Mayer et al and as other markers are conventionally used for in cancer treatment, does not mean that the marker will be useful as a predictor of metastasis by detection of the marker in an as yet

nonmetastasized (primary) cancer, as claimed in claim 1, as amended.

Kim et al

Kim et al discloses the results of fusing *in vitro* two tumor cell lines with syngeneic thymocytes and activated macrophages to test the notion that metastatic potential of tumor cells is acquired via somatic hybridization with host immune cells and not by the progression of transforming process alone. Metastasizing tumors were produced and T-cell and/or macrophage associated products were found on the surfaces thereof, it being stated that this indicates that lymphoid cell-associated molecules may be directing the metastatic route. Kim et al also discloses that fusion of the tumor cells with either syngeneic thymocytes or activated macrophages alone had not yielded metastasizing cells.

The tumors discussed in Kim et al are artificially produced and would thus be expected to have products of the thymocytes and macrophages from which they were fused. However, this does not suggest (and Kim et al does not suggest) that natural human tumors would have such products. Moreover, this does not suggest (and Kim et al does not suggest) that the presence of such products would be predictive of metastatic potential of a primary tumor.

Summary

Neither Barbera-Guillem et al, Mayer et al, Kim et al, or any other of the references of record, whether taken together or individually, discloses, teaches, or suggests a method of predicting the lymphotropic metastatic potential of a solid non-lymphoid primary tumor wherein the percentage of cells of each of representative samples of the tumor which express one or more of the products TCRβ, CD3, CD4, CD8, and ZAP-70 is determined,

wherein the metastatic potential of the tumor is predicted to be low when no tumor cells in all of the samples are detected to express any of the products and predicted to be high when a high percentage of the tumor cells in at least one of the samples are detected to express any of the products, as claimed in claim 1, as amended, so that a prediction may be reliably made as to whether a primary (localized) tumor will (in the future) metastasize to distant sites. Therefore, it is respectfully submitted that claim 1, as amended, is not anticipated by and is unobvious over the prior art and therefore patentable.

It is therefore respectfully submitted that this application is in condition for allowance, and such is respectfully requested. If it would aid in advancing this application to issue, the Examiner is respectfully urged to call the undersigned attorney for Applicant at the number below.

Respectfully submitted,

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Enclosure

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